

SCORING OF TETANUS IMMUNITY IN MICE¹

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In a recent study associated with the development of a method of assaying tetanus toxoid in minimally sensitized mice (1), it became necessary to adopt a measuring technic which is capable of gauging accurately the immunity of the animals under test. The quantal response method, using survival or death of the animal as the gross end point, was considered inadequate for this purpose. It was decided that we should resort to the use of graded response method, by which better gradation of the group response is rendered possible by assigning different scores according to the severity of signs developed.

Two scoring systems are available for measuring the immunity in experimental tetanus, *i. e.*, the original system of Ipsen (2) and the modified system of Wada *et al.* (3). It will be noted below that only slight difference exists between the two scoring systems, in that different scores are assigned to those animals died at the 3rd or 4th day after toxin challenge. Since such minute difference might nevertheless affect the efficiency of the scoring systems concerned under different experimental conditions, some of our own experimental data were therefore analyzed by both methods for comparison. The protocols selected for analysis were those which contained at least one animal died at the 3rd or 4th day after challenge. The results obtained constitute the basis of this report.

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MATERIALS AND METHODS

Mice

Female mice of BALB strain were used. At the time of primary immunization, the mice weighed generally 13-17 g with the exception of experiments 1-6, in which about 1/3 to 1/4 of the animals weighed 10-12 g. Balanced animal weight between each group in any experiment was achieved by adequate planning.

Tetanus Toxoid

Both LE 202 and WHO were plain, monovalent toxoids. 1169-1 was a diphtheria-pertussis-tetanus combined vaccine without adjuvant. Both 43J and TRC were Al(OH)₃ adsorbed products, the former was a diphtheria-tetanus combined vaccine while the latter was diphtheria-pertussis-tetanus combined.

Scoring

The scoring scales recommended by Ipsen (2) and Wada *et al.* (3) are as follows:

Ipsen					
Days dead				Signs of survivals	
	1-2	3-4	5-7	at 7th day	
				+	-
Score	0	2	3	4	6

Wada <i>et al.</i>					
Days dead				Signs of survivals	
	1-2	3	4-7	at 7th day	
				+	-
Score	0	1	3	4	6

Exp. No.	Toxoid	Serial dose interval, -fold	No. of mice	Challenge dose, MLD	B-C interval, days	Result after challenge							<i>b</i>		<i>s</i> ²		<i>F</i> *		
						Days dead													Signs of survivals at 7th day
						1	2	3	4	5	6	7	+	—	Ipsen	Wada	Ipsen	Wada	Ipsen
1	LE 202	3.3	10 10 10 10	5	6	1 3 2 6				1 1		9 5 6 2	2.077	1.999	6.14	6.08	0.95	1.04	
2	LE 202	3.3	10 10 9 9	5	6	2 1 5	1					4 1 1	5 8 7 3	1.280	1.238	4.75	5.16	2.71	2.35
3	LE 202	3.3	9 10 10 10	5	6	2 2 9 9	1 1					2 1	4 6 1 1	2.607	2.52	5.03	5.31	3.28†	2.78
4	LE 202	3.3	10 10 10 10	5	7	1 1 1 5	1 2 1	1				1 1 2	7 6 8 2	1.344	1.306	5.10	5.63	2.91	2.91

TABLE I *Continued*

Exp. No.	Toxoid	Serial dose interval, -fold	No. of mice	Challenge dose, MLD	B-C interval, days	Result after challenge								<i>b</i>		<i>s</i> ²		<i>F</i> *		
						Days dead							Signs of survivals at 7th day							
						1	2	3	4	5	6	7	+	—	Ipsen	Wada	Ipsen	Wada	Ipsen	Wada
5	LE 202	3.3	10 10 10 10	5	7		2 3 7 6	1 1 2					1 2 3 2	2.098	2.199	6.97	7.07	0.32	0.22	
6	LE 202	3.3	9 9 9 8	5	7						1	1 2 1	8 6 5 6	1.021	1.049	4.04	4.19	0.63	0.76	
7	LE 202	3.3	9 10 10 9	5	7			3 1 5 6	1 1 1		1 1	1 2 3	4 2 1	0.694	1.295	2.65	2.94	0.81	0.37	
8	LE 202	3.3	9 9 10 9	5	13			2 1 3 3	1 1 3 1			1 1	6 6 3	2.296	2.452	3.84	4.41	1.12	1.27	
9	LE 202	3.3	10 10 10 10	5	7		1 4 3 2	1 2 4	2 2 1		1 1	1 2 2	5 2 1 2	0.811	1.204	4.80	5.11	0.23	1.39	
10	LE 202	3.3	10 10 10 10	5	10			1 3 2			1	1 2 1	10 8 5 2	2.161	2.437	2.41	3.31	0.63	0.45	
11	LE 202	3.3	10 10 10 10	5	13			1 2 1	1 1 3	1 1			10 8 5 3	1.895	1.996	3.33	3.84	0.31	0.15	
12	LE 202	3.3	12 12 12 12	5	10		2 5 4	4 4				2 2 1 1	10 4 6 2	2.169	2.301	4.92	5.32	0.92	1.47	
13	LE 202	3.3	10 10 10 9	5	10		1 2 1 4	3 1 6 3	1			3 1 1	5 4 2 1	1.416	1.612	4.93	5.72	0.22	0.51	
14	LE 202	3.3	9 10 10 9	5	14			1 5 3 2 4	3 2 2		1	1 1	3 3 1 1	1.097	1.051	3.36	3.89	1.51	1.95	
15	LE 202	3.3	9 10 10 9	20	10		3 5 6	1 1		1		3 1 3 1	6 5 1 1	2.291	2.359	4.83	4.84	1.21	1.32	

TABLE I *Continued*

Exp. No.	Toxoid	Serial dose interval, fold	No. of mice	Challenge dose, MLD	B-C interval, days	Result after challenge								b		s ²		F*	
						Days dead							Signs of survivals at 7th day						
						1	2	3	4	5	6	7	+	-	Ipsen	Wada	Ipsen	Wada	Ipsen
16	LE 202	5	8 8 8 8	5	10	1 2 4 4	1 4 2 4		1			1 1	5 1 1	1.519	1.645	3.96	4.11	0.66	0.99
17	LE 202	5	8 8 8 7	20	10	2 2 5 7	2 2 1			1		6 5 1	2.343	2.384	5.13	5.07	0.81	1.02	
18	LE 202	5	10 10 10 9	5	10	1 3 3 4	2 2 1				1 2 1	7 6 4 2	1.351	1.454	4.05	5.2	0.47	0.49	
19	LE 202	5	10 10 10 10	5	9	1 4 7	3 2 1		1 1	1	1	7 4 2 1	1.245	1.488	2.94	3.83	0.72	0.21	
20	LE 202	5	10 10 10 10	10	10	1 3 5 7	1 1		1		1 2 1 1	8 5 3 1	2.598	2.618	5.99	6.04	0.03	0.06	
21	LE 202	5	9 10 10 9	10	11	1 2 2 3	1 1 1		1		8 7 8 3	1.054	1.117	4.40	5.28	0.54	0.78		
22	LE 202	5	10 10 10 10	10	10	3 2 7	1 1 1		2	1 2	7 5 5 1	1.717	1.731	4.94	5.19	2.69	1.85		
23	WHO	5	11 11 11 11	20	10	1 3 4 4	1 1 1			3	11 9 4 6 4	1.304	1.298	5.39	5.62	0.47	0.52		
24	43J	5	9 9 9 9	15	10	3 6	2	1 1	1	1	8 7 5 1	2.178	2.272	3.83	3.76	1.59	1.97		
25	TRC	5	8 9 9 8	15	10	2 2	1 2		1	1 3 1	8 8 2 3	1.572	1.695	3.02	3.35	1.97	1.67		
26	43J	10	14 13 15 14	15	10	5 7 8	2 3 5	1			14 5 5 1	1.503	1.621	4.53	4.61	2.55	0.78		

TABLE I *Continued*

Exp. No.	Toxoid	Serial dose interval, -fold	No. of mice	Challenge dose, MLD	B-C interval, days	Result after challenge								b		s ²		F*				
						Days dead							Signs of survivals at 7th day									
						1	2	3	4	5	6	7	+	-	Ipsen	Wada	Ipsen	Wada	Ipsen	Wada		
27	TRC	10	15 14 14 14	15	10			2 4 7			5 4	1			2 1	15 10 4 2	1.512	1.627	3.77	3.96	18.17 ^{††}	0.92
28	TRC	10	10 11 10	10	10			2 8	2 2				1	1	10 5	2.799	2.899	2.38	2.51	0.82	0.54	
29	1169-1	10	10 11 10	10	10		1 5 6	1 4 2	1 1 1				1	1 1	5 1 1	1.45	1.5	4.18	4.29	1.73	2.91	

* The P values of *P* are >0.05 except that with † is <0.05 and that with †† is <0.01.

the linear relationship.

Computation results are given in detail in Table I. For the purposes of concise presentation and conspicuous contrast, however, the lengthy original figures in Table I can be condensed by processes of grouping and subsequent breaking-down. Such a condensed rearrangement of the original data is given in Table II.

It is evident from Table II that so far as the regression coefficient *b* is concerned, the modified scoring system of Wada *et al.* is definitely better than the original system of Ipsen. Out of a total of 29 experiments, the former

system yields steeper slopes in 23 experiments while the latter in the remaining 6 only. The extent of difference in slope values obtained by the two scoring systems also gives suggestive clues. In the minority of cases where Ipsen's system yields steeper slopes, the difference is invariably slight (<0.1). In contrast, in about 2/3 (actual figure is 15/23) of the cases where the scoring system of Wada *et al.* gives a steeper slope, the difference exceeds 0.1. This is particularly significant in experiments 7 and 9, where the distribution pattern of animals died at the 3rd or 4th day after challenge is such

TABLE II
Extent of difference of b, s² and F obtained on the basis of scoring systems of Ipsen and Wada et al.

Extent of difference Favorite	Regression coefficient <i>b</i>										Total
	<0.1	0.1-0.19	0.2-0.29	0.39	0.6						
Ipsen	6										6
Wada <i>et al.</i>	8	11	2	1	1						23
Extent of difference Favorite	Average error variance s ²										Total
	<0.1	0.1-0.19	0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59	0.79	0.8-0.89	0.89	1.15	
Ipsen	3	6	4	2	2	4	1	2	1	1	26
Wada <i>et al.</i>	3										3
Extent of difference Favorite	Variance ratio <i>F</i>										Total
	<0.1	0.1-0.19	0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59	0.84	1.18	1.77	17.25	
Ipsen	4	4	3	2	1	2		1			17
Wada <i>et al.</i>		3	1	2	1	1	1		1	1	11

that it gives differences of slope values of 0.60 and 0.39 respectively, resulting in slope ratios of about 2:1 and 3:2, which are by no means negligible, in favor of the scoring system of Wada *et al.* Since the superiority of the system of Wada *et al.* in yielding steeper slopes is not correlated with any of the experimental factors involved in this study, it is concluded that it represents the true, instead of biased, result.

The situation is completely reversed however, when the precision of regression coefficient is taken as the criterion for comparison. Here the scoring system of Ipsen proves to be decisively better than that of Wada *et al.* Out of 29 experiments the former system gives smaller average error variance in 26, while the latter in 3 only. Moreover, in the mere 3 cases where the system of Wada *et al.* gives smaller values of s^2 , the extent of difference is invariably diminutive. On the other hand the difference is apt to be significantly large when Ipsen's system gives smaller s^2 values, *e. g.*, experiments 10, 13, 18, 19 and 21. Again, the available data indicate that the ability of Ipsen's system to give smaller s^2 is not determined by any of the experimental factors *per se*.

The overall figures of variance ratio F listed in Table I indicate that the departure from linearity is generally statistically insignificant under the conditions of the experiment. The series of relatively big figures of F , approaching 0.05 probability values for the distribution of variance ratio, in experiments 2, 3 and 4 are likely artifacts due to the trial use of small initial weight of mice, weak challenge dose and/or short interval between secondary immunization and challenge.

So far as the point of linearity testing is concerned, the two scoring systems give agreeable results in the majority of cases. In 26 experiments out of 29, the probability of departure from linearity computed on the basis of the two different systems shows immaterial differences which are statistically negligible (Table I). The frequency distribution of the extent of difference of F values obtained by the two systems is also comparable (Table II). However, the differences shown in experiments 9, 26, 27 are sufficiently large to cause attention, especially in experiment 27, where the statistically significant departure from linearity by Ipsen's system stands in contrast to the insignificant figure by the scoring system of Wada *et al.* The reason for the emergence of these extraordinary cases is not apparent. Additional data are needed before definite conclusions could be rightly drawn.

The available findings indicate therefore, that neither of the two systems is flawless by themselves, and that the choice must depend on the balance of experimental evidences. If the experimenter aims to get a steeper dose-response slope at the expense of the precision of slope, the scoring system of Wada *et al.* is preferable. Contrarily, if the situation calls for enhancing the precision of slope while its steepness is of less concern, then Ipsen's system is certainly recommended.

In a comparative study on bio-assay of various forms of tetanus toxoid (1), the senior author has compared the statistical efficiency between different methods of standardization: guinea pig primary (GPP), mouse primary (MP) and mouse minimally sensitized (MSM). With the use of the scoring scale of Wada *et al.*, the regression coefficient b and the average error variance for the total doses s^2 obtained by the above mentioned methods were analyzed. The following arithmetic means were obtained:

	GPP (6*)	MP (8)	MSM (16)
b	4.857	3.403	1.594
s^2	1.77	2.95	5.47

* No. of estimates from which the arithmetic means were obtained.

It is apparent from the above that GPP is the best method statistically in that it yielded the steepest slope with a greatest precision, MP ranks next, and MSM is least satisfactory. It becomes clear therefore that in both GPP and MP methods, where the steepness of slope is already acceptable, it is preferable to use Ipsen's scoring system which tends to improve further the precision of slope. In MSM however, it pays to use the modified system of Wada *et al.*, because the situation here is such that any improvement in the steepness of slope would count. In this case one probably would not gain too much by trying to minimize somewhat the error of the slope, because it is on immunological instead of statistical soundness MSM finds its basis, a problem which is beyond the scope of this discussion and is going to be treated fully in the coming paper (1).

SUMMARY

Twenty nine experiments of secondary immunization with graded doses of tetanus toxoid in mice were scored separately by the scales of Ipsen and Wada *et al.* The regression coefficient, the average error variance for the total doses, and the probability of deviation from the linear

relationship were computed on the basis of the two systems and compared.

It was shown that the original scoring system of Ipsen yielded, in the majority of cases, flat slopes with smaller average error variances. The reverse was true of the modified system of Wada *et al.* The two systems gave comparable figures for the probability of departure from linearity.

It was concluded that neither system is flawless, and that the choice of the scoring systems depends on the balance of experimental evidences.

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